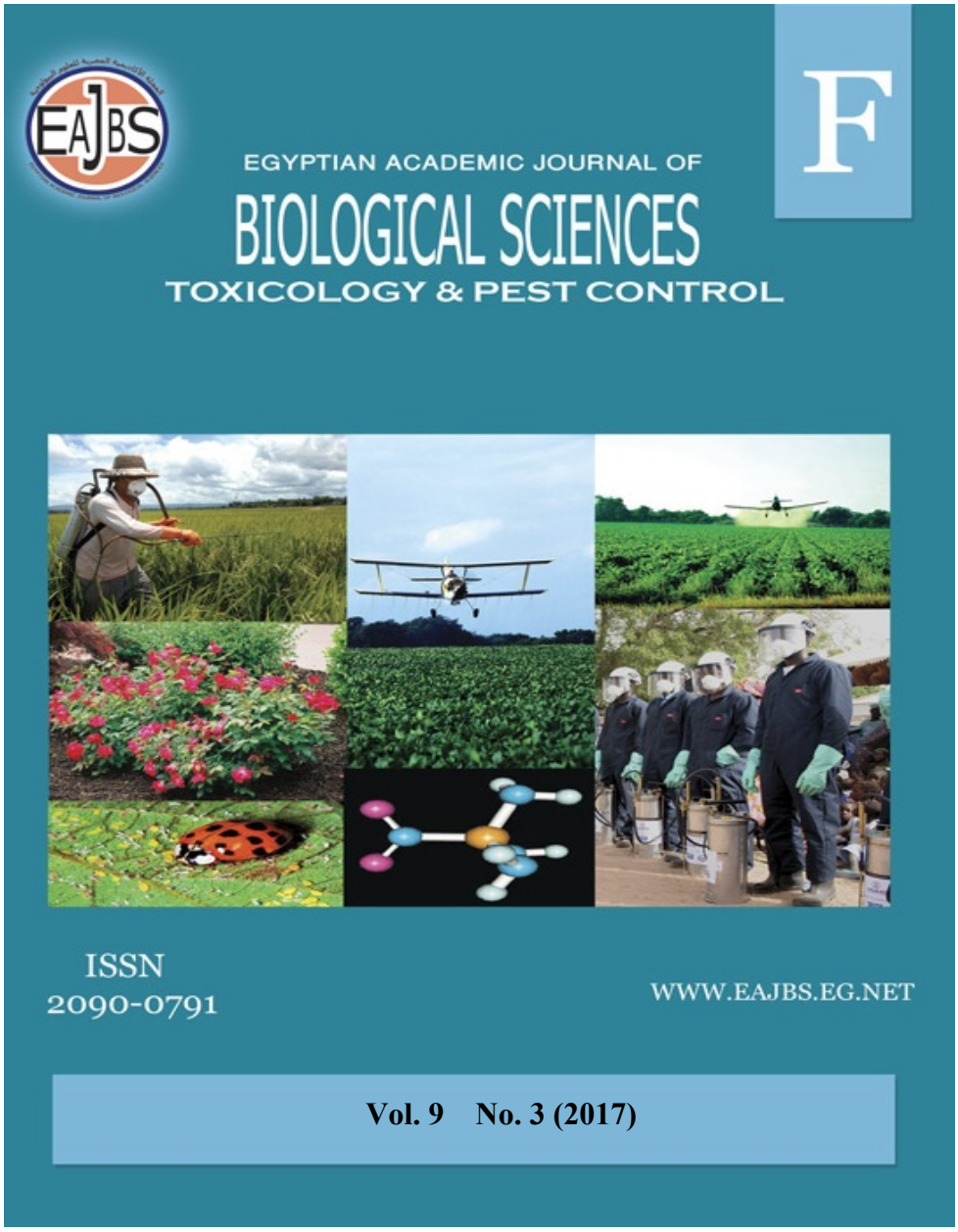

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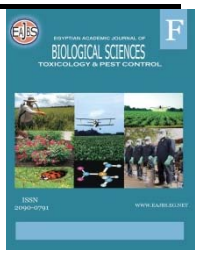

Insecticidal Activities of Some Actinomycete Strains Isolated from the Egyptian Sinai Soils

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ABSTRACT

Seventy three pure actinomycete colonies were isolated from 48rhizospheric soil samples revealing different locations in Sinai. These isolates were subjected for measurement of their insecticidal activities against the greater wax moth *Galleria mellonella* L. of them seven isolates (S6, S13, S16, S23, S27, S35 and S36) were found as the most potent and were chosen for detailed toxicological studies. Their LC_{50} values were 25.23, 36.80, 55.96, 52.02, 54.19, 54.52 and 32.88 mg/ml, respectively. The most potent isolate (S6) was isolated from the rhizosphere of *Tamarix nilatie* plants grown in a sandy soil at El-Tor area and taxonomically was identified as *Streptomyces lavendulae*.

INTRODUCTION

The Greater wax moth (*Galleria mellonella* L.) is a highly destructive insect that attacks and destroys beeswax combs especially in weak colonies or during storage. The moth itself is not a problem. Damage is caused only by the caterpillars, which feed on combs, propolis, pollen, larval skins and other protenaceous matters. The larvae tunnel into the combs leaving them a mass of webs and debris (USDA 1981).Some beekeepers store their combs in moth-tight cupboard or keeping them in sealed polythene bags or wrapping them in newspapers. Others use chemical fumigants in treating wax moth such as paradiclorobenzene (PDB), ethylene dibromide, phosphine, acetic acid 80%, sulphur or naphthalene (moth balls). Most of these chemicals even they are active against moths and larvae, they are not effective against eggs and pupae. In addition they contaminate honey and remain as a residue in the wax. Some tried to use biological control methods such as using DIPEL,the bacterium *Bacillus thuringiensis* product which is widely used to control caterpillars but it is not fully effective against the wax moth. (USDA 1981, MAAREC 2000, Someville 2007 and the British Beekeepers Association 2012). Placing a layer of tobacco leaves or other herbs between boxes of combs in storage gave acceptable results. Elbehery *et al.* (2016) tested Neem Azal- T/S, in the laboratory and under semi-field conditions.

The use of natural products obtained from plants and microorganisms in biological control of pests has been adopted recently in agriculture (Dong and Zhang, 2006, Hu *et al.,* 2007). The actinomycetes and their bioactive metabolites have shown to possess antimicrobial, cytotoxic, plant growth promotory, antiviral, antioxidant, insecticidal, antiprotozoal, anthelmintic, enzyme inhibitor, plant growth promoting and herbicidal agents (Alam *et al.,* 2012; Yokomizo *et al.,* 1998). The aim of the present study was to investigate the insecticidal activity of some soil actinomycetes isolates against the greater wax mothand the ability to use them as environmentally and friendly alternatives for the extensive use of chemical pesticides in plant protection programs.

MATERIALS AND METHODS

Sample collection and preparation

Forty eight soil samples were collected from fifteen different localities distributed through South and North Sinai Governorates, Egypt. The samples were collected aseptically from a 10-15 cm depth in clean plastic bags with a sterile spatula. The samples were immediately brought to the laboratories of the Plant Protection Department, DRC. Soil samples were air-dried and were sieved through a 2mm sieve. To reduce the vegetative bacterial cells and allow the spores of actinomycetes to survive the sieved soils were mixed before plating with Ca CO_3 in a 10:1 (w/w) ratio.

Isolation of actinomycetes colonies:

One gram of the prepared soil was suspended with 10 ml of sterile distilled water and incubated at room temperature (25 ± 2°C) for 1h on a rotary incubator shaker with vigorous shaking. Soil suspension (100 µl) was spread on starch nitrate agar (SCA) Petri-dishes and incubated at 30°C for 4 days. Colonies of actinomycetes were picked up using sterile toothpicks, placed onto SCA plates and incubated for 7 days. Pure colonies of actinomycetes were then subcultured onto SCA slants and incubated for 7 days at 30°C (Sandeepa and Menaka 2014).

Preparation of actinomycete filtrates

Actinomycetes filtrates were prepared by cutting 3 discs (9 mm in diameter) of pure actinomycetecolony , inoculated in a 250 ml Erlenmeyer flask containing 100 ml of a liquid medium (starch nitrate broth), and incubated on a shaker (200 rpm) at 30 °C. for 7 days (Walker *et al*., 1966). At the end of the incubation period, the culture broth was centrifuged at 15,000 rpm (1260 g) for 20 min.and the supernatant was stored at 4 °C. until used.

Test insects:

Larvae of the greater wax moth, *Galleria mellonella* L. (Lepidopetra: Galleridae) were successfully mass reared in Plant Protection Department laboratories, Desert Research Center on an artificial diet described by (Metwally *et al*., 2012).

Screening of actinomycete filtrates for their insecticidal activity :

For preliminary screening studies actinomycete culture filtrates were used directly without dilution. Three ml of each actinomycete filtrate were mixed with10 gm of the greater wax moth artificial diet and were stirred homogeneity before placing in 250 ml clearly plastic cups. Cups were held uncapped for half an hour to allow drying, then five 3rd instar larvae of *G. mellonella* were placed into each cup using fine hairbrush and capped with tiny bored cover. In the control treatment, 3 ml of free media of starch nitrate broth were added and mixed with the larval diet. Each treatment was replicated ten times. The cups were examined after 10 days and the isolates which cause 25% or more larval mortality were considered as

an active isolates. These active isolates showed a second test by the same way where the cups were examined after 3, 6, 9, 12, and 15 days to determine the rate of larval death, and follow-up to pupation, and adult development.

Dose-response bioassay:

The most potent isolates were further investigated for more detailed toxicological studies. Chosen filtrates were dried under reduced pressure and series of concentrations (100, 50, 25 and 12.5 mg/ml) were prepared and were tested as above. Mortality and abnormality were observed. Larval mortality data were subjected to probit analysis (Finney, 1971) after correction for natural mortality observed in the controls (Abbott, 1925) and the LC_{50} and LC_{90} values were recorded. Relative toxicity index was used to compare between isolate activities.

Relative Toxicity Index (LC_{50}) = 100*(the lowest LC_{50} value / the desired LC_{50} value).

Relative Toxicity Index (LC_{90}) = 100*(the lowest LC_{90} value / the desired LC_{90} value).

Identification of the most potent actinomycete isolate:

The most active actinomycete isolate among the tested actinomycete isolates was subjected for further studies concerning its identification.It was conducted according to recommended international Key's given in Bergey's Manual of Determinative Bacteriology 8thedition (Buchanan and Gibbsons, 1974), Bergey's Manual of Systematic Bacteriology, Vol. 4 (Williams*et. al.,* 1989) and Bergey's Manual of Determinative Bacteriology, 9th edition (Hensyl, 1994).

RESULTS AND DISCUSSIONS

Screening of actinomycete filtrates for their insecticidal activity:

Primary Screening:

Forty eight soil samples were collected from 15 sites in north and south Sinai. Soil types, associated plants and number of isolates from each site were tabulated in Table (1). From these 48 collected soil samples, seventy three actinomycete isolates were obtained and purified. Actinomycete isolates were subjected to primary screening for their insecticidal activities against the greater wax moth *G. mellonella* third instar larvae. Only twenty three isolates (represented in Table 2) exhibited high biological activities against the tested larvae (≥ 25% larval death). On the contrary twenty six actinomycete isolates (35.62%) failed to exhibit any biological activity, while the remainder isolates (32.88%) were exhibited moderate to slight effects (<25% larval death).

These 23 active isolates undergo another test by the same way to determine larval death, and follow-up to pupation and adult development. As shown in Table (2) of these 23 only four isolate filtrates (S6, S16, S23 and S35) were highly effective causing 80% or more larval death and giving the least numbers (10-18) of emerged moths. Three isolates S13, S27 and S36 exhibited 76 - 78% mortality. Another thirteen isolates (S2, S3, S4, S5, S7, S8, S20, S21, S22, S28, S40, S41 and S46) revealed 50-70% larval mortality while the remainder isolates (S1, S14 and S19) showed mortalities ranged from 42 to 48%. On the other hand 13 isolates (S 3, S 4, S 5, S 14, S 19, S 20, S 21, S 22, S 23, S 27, S 35, S 40, S 41 and S 46) caused slight harmful effects on the pupal stage while the others were safe. Emerged moths were ranged between 10% for S6 isolate and 54% in S14 isolate. Untreated control treatment showed 96% adult emergence with 4% normal larval death.

Table 1: Collected soil samples, locations, soil type and associated plants

Governorate	No.	Location	# Isolates	Soil type	Associated plants	Plant scientific name
South Sinai	1	Hamamat pharoun	2	Sandy	Capparis Gharqad الغردق	*Capparis spinosa* *Nitraria retusa*
	2	El Tor	4	Sandy	Tamarix, prosopis, dates	*Tamarix nilotica* *Prosopis juliflora* *Phoenix dactylifera*
	3	Ras Mohammed	6	Sandy- loam	Mangrove	*Avicennia marina*
	4	Nabq reserve	6	Sandy	Mangrove, Arak	*Avicennia marina* *Salvadora persica*
	5	Al Ruizah	2	Sandy- loam	Mangrove	*Avicennia marina*
	6	Dahab	6	Sandy	Arak Dates Aqool	*Salvadora persica* *Phoenix dactylifera* *Alhagi maurorum*
	7	Abu Gallum reserve	8	Clay Sandy -loam Sandy	Capparis Fig, lemon, guava, egg plants,	*Capparis spinosa* *Ficus carica* *Citrus* spp *Psidium guajava Solanum melongena*
	8	Wadi Wateir	5	Sandy - loam	Lemon, Dates	*Citrus* spp *Phoenix dactylifera*
	9	Sant Catherine	4	Sandy-loam	Olives, Carob, Tobacco, been	*Olea europaea* *Ceratonia siliqua*, *Nicotiana tabacum* *Phaseolus vulgaris*
	10	Wadi feiran	7	Sandy- loam Clay- loam	Dates alfalfa	*Phoenix dactyliferaMedicago sativa*
North Sinai	11	El-Quseima	5	Sandy -loam Sandy	Olives Dates	*Olea europaea* *Phoenix dactylifera*
	12	El-Hasana	4	Sandy	Olives	*Olea europaea*
	13	Bir al- Abd	5	Sandy	Olives Dates	*Olea europaea* *Phoenix dactylifera*
	14	Al Arish	5	Sandy -loam	Olives Dates egg plants	*Olea europaea* *Phoenix dactylifera Solanum melongena*
	15	Rafah	4	Sandy -loam	Olives	*Olea europaea*
Total			**73**			

Table 2: Insecticidal activity of Actinomycete filtrates against different stages of *G. mellonella*

No.	Soil Sample	Location	Plant	% mortality in			% emerged adults
				larvae	pupae	Total	
1	**S 1**	Hamamat Pharoun	*Nitraria retusa*الغردق	48	0	48	52
2	**S 2**			60	0	60	40
3	**S 3**	El Tor	*Tamarix nilotica*	56	2	58	42
4	**S4**			66	2	68	32
5	**S 5**			66	4	70	30
6	**S 6**			**90**	**0**	**90**	**10**
7	**S 7**	Ras Mohammed	*Avicennia marina*	62	0	62	38
8	**S 8**			58	0	58	42
9	**S 13**	Nabq reserve	*Salvadora persica*	**76**	**0**	**76**	**24**
10	**S 14**			42	4	46	54
11	**S 16**			**82**	**0**	**82**	**18**
12	**S 19**	Al Ruizah	*Avicennia marina*	48	8	56	44
13	**S 20**			60	2	62	38
14	**S 21**	Dahab	*Salvadora persica*	66	2	68	32
15	**S 22**		*Phoenix dactylifera*	52	4	56	44
16	**S 23**		*Alhagi maurorum*	**80**	**2**	**82**	**18**
17	**S 27**	Abu Gallum reserve	*Ficus carica*	**76**	**2**	**78**	**22**
18	**S 28**		*Citrus* spp	70	0	70	30
19	**S 35**	Wadi Watir	*Solanum melongena*	**82**	**2**	**84**	**16**
20	**S 36**		*Phoenix dactylifera*	**78**	**0**	**78**	**22**
21	**S 40**	Saint Catherine	*Olea europaea*	62	4	66	34
22	**S 41**		*Phaseolus vulgaris*	50	8	58	42
23	**S 46**	Wadi feiran	*Medicago sativa*	58	6	64	36
24	**Control**			4	0	4	96

In order to determine the rate of larval death cups of the promising active isolates were examined after 3, 6, 9, 12, and 15 days and the results were tabulated in Table (3) which showed that the tested isolates caused considerable mortalities to *G. mellonella* larvae throughout the period of the test and that the rate of larval death was time dependent. The rate of larval death increased as the time elapsed. This is clear in Fig. (1) which represent the mean number of larval mortality of the seven active isolates at the candidate days. According to that the cumulative larval mortalities increased with time and finally the rate of adult emergence will decreased.

Table (3): Mortality rates of tested isolated filtrates on *G. mellonella* larvae.

Isolates	% of larval mortality at candidate intervals(days)					Total larval mortality
	3rd	6th	9th	12th	15th	
S 6	10	14	16	24	26	90
S 13	8	12	14	18	24	76
S 16	6	14	20	20	22	82
S 23	8	18	16	18	20	80
S 27	8	18	14	16	20	76
S 35	10	14	18	22	18	82
S 36	6	14	18	20	20	78
Mean	**8.0**	**14.9**	**16.6**	**19.7**	**21.4**	
Accumulative larval mortality	**8.0**	**22.9**	**39.4**	**59.1**	**80.6**	

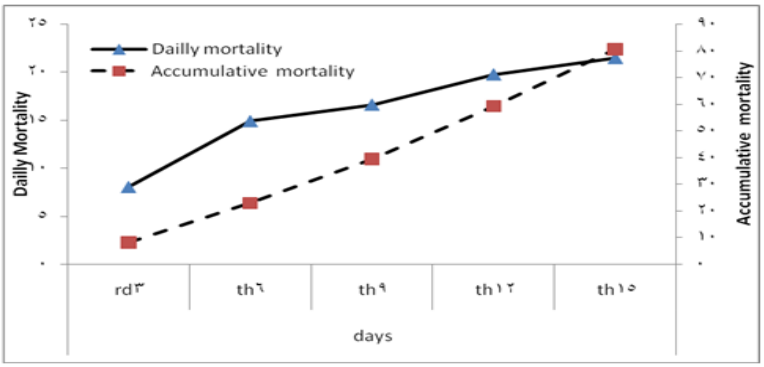

Fig. 1: Accumulative mean larval mortalities and mean mortality rate of tested isolated filtrates on *G. mellonella* larvae

Dose – response bioassay

The filtrates of these 7 most active and promising isolates were further investigated for more detailed toxicological studies. The results of these studies were tabulated in Table (4) and were illustrated in Figure (2) which clearly proved that the toxic responses were concentration dependant and that the S 6 isolate was the most potent isolate. At any of the tested concentrations it gave the highest percentage of larval mortality. That was revealed in its lowest LC_{50} and LC_{90} values (25.83 and 145.31 mg/ml, respectively). Isolates S 36 and S13 came in the following steps with 35.30 and 43.04 mg/ml medium lethal concentration values, respectively, while S 27, S 35 and S16 isolates were the least effective with LC_{50} values 54.16, 54.95 and 55.95 mg/ml, respectively. Dose-response curves among chemicals can offer information about the chemicals as well. The more potent the chemical, the less it takes to kill. The dose-response curves for these actinomycete isolates were illustrated in Fig. (2).A steep curve

that begins to climb even at a small dose suggests a chemical of high potency that any little change in its concentration causes a noticeable change in its response and that a relatively flat slope suggests that the effect of an increase in dose is minimal. This is clear in S 13 curve which was the flattest line. Concerning to its LC_{50} value (43.04 mg/ml), it was the third between the tested isolates but it needs high increase in filtrate concentration to reach its LC_{90} level (1001.40mg/ml) which had the highest value between the tested isolates. These results were reflected in their relative toxicity index values. For the LC_{50}, the relative toxicity indexes were 73.17 and 60.01 for S 36 and S13 isolates as compared with that for the S 6 isolate. The values were 46.16, 51.11, 47.69 and 47.00 for S 16, S 23, S 27 and S 35 isolates, respectively.

Table 4: Larvicidal activity of different actinomycete filtrates against *G. mellonella* larvae

Isolate / Conc.(mg/ml)	Larval mortality (%)						
	S6	**S13**	**S16**	**S23**	**S27**	**S35**	**S36**
12.5	29.90	31.96	13.40	11.34	11.34	9.28	21.65
25	50.52	40.21	23.71	25.77	23.71	23.71	38.14
50	64.95	50.52	42.27	42.27	46.39	42.27	54.64
100	85.57	64.95	71.13	77.32	71.13	73.20	83.51
Trend line equation	y = 1.706x + 2.590	y = 0.936x + 3.469	y = 1.832x + 1.797	y = 2.102x + 1.418	y = 1.967x + 1.589	y = 2.108x + 1.330	y = 1.891x + 2.072
R2	0.988	0.985	0.978	0.973	0.996	0.991	0.971
Slop	1.71	0.94	1.83	2.10	1.97	2.11	1.89
Intercept	2.59	3.47	1.80	1.42	1.59	1.33	2.07
LC_{50} (mg/ml)	25.83	43.04	55.95	50.53	54.16	54.95	35.30
LC_{90} (mg/ml)	145.31	1001.40	279.54	205.28	242.22	222.31	167.71
Relative Toxicity Index (LC_{50})	100.00	60.01	46.16	51.11	47.69	47.00	73.17
Relative Toxicity Index (LC_{90})	100.00	14.51	51.98	70.79	59.99	65.36	86.64

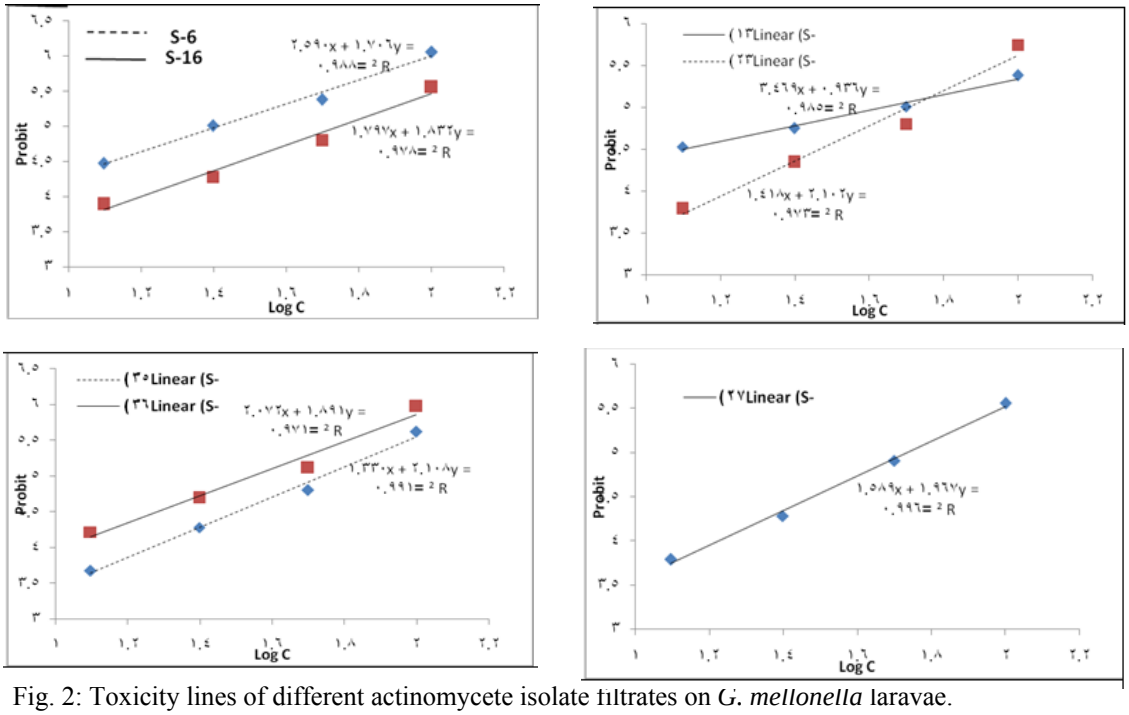

Fig. 2: Toxicity lines of different actinomycete isolate filtrates on *G. mellonella* laravae.

Identification of the most potent actinomycete isolate:

The (S 6) isolate, the most active actinomycete isolate among the tested actinomycete isolates, was subjected for further studies concerning its identification. Morphological, physiological and phylogenetic analysis of 16S rRNA gene; in addition to biochemical studies and culture characteristics were carried out. On the basis of the accumulated characteristics of the S 6 actinomycete isolate and consulting the recommended International Key's of Bergey's Manuals for identification of actinomycetes (1974, 1989 & 1994), it was found that the actinomycete isolate S 6, was more similar to *Streptomyces Lavendulae*.

The present results are, however, in accordance with several results performed with actinimycetes and other insect species. They are in harmony with Osman *et al.* (2007) who isolated fifteen local isolates of Streptomyces from different soils and geographical areas in Egypt and evaluated their efficacy as antagonistic agents against the cotton leaf worm, *Spodopetra littoralis*. Four of these Streptomycete isolates namely S05, S08, S10 and S15 were recorded as the most effective as they showed 80, 100, 70 and 80% mortality, respectively. Many researchers reported that actinomycetes play an important role in the biological control of different insects species through the production of insecticidal active compounds against the house fly *Musca domestica* (Hussain *et al.*, 2002), *Culex quinquefasciatus* (Sundarapandian *et al.*, 2002), *Drosophila melanogaster* (Gadelhak *et al*., 2005), *Anopheles* mosquito larvae (Dhanasekaran *et al.*, 2010) and *Culex pipiens* mosquito larvae (El-Khawagah *et al*., 2011).

Detailed and more research studies are needed in this point to test these products in hives and during comb storage. These previous studies light the scope on the insecticidal activities of actinomycetes metabolites and the ability to use them as alternative control agents in integrated pest management programs. They are safe and environment friendly products.

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ARABIC SUMMERY

النشاط الإبادى الحشرى لبعض سلالات الأكتينومايسيت المعزولة من تربة سيناء المصرية

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١- قسم وقاية النبات - مركز بحوث الصحراء – المطرية – القاهرة – مصر

٢- قسم النبـات والميكـروبيولوجي - كلية العلـوم (بنين) - جامعة الأزهـر– القاهرة – مصر

تم الحصول على ٧٣ عزلة نقية من الأكتينومايسيت تم عزلها من ٤٨ عينة تربه من منطقة الرايزوسفير ممثلة لمختلف الأماكن بسيناء. هذه العزلات تم اختبار نشاطها الإبادى الحشرى ضد دودة الشمع الكبرى *Galleria mellonella* L. العزلات السبع التى اظهرت اعلى كفاءة , (S6, S13, S16, S23, S27, S35 S36) تم اختيارها لإجراء دراسات سمية تفصيلية فكانت الجرعة القاتلة ل ٥٠% من الحشرات المختبرة كالتالى ٢٥,٢٣ – ٣٦,٨٠ - ٥٥,٩٦ – ٥٢,٠٢ – ٥٤,١٩ – ٣٢,٨٨ ملجم/مل بالترتيب. العزلة S6 الاعلى كفاءة كان قد تم عزلها من ريزوسفير نبات التامريكس *Tamarixnilatie* النامى بتربة رملية بمنطقة الطور و تم تعريفها على انها ستربتومايسيس لافينديولا *Streptomyces lavendulae*.